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PRINCIPAL INVESTIGATOR: Craig J. Ceol, Ph.D.

CONTRACTING ORGANIZATION: University of Massachusetts, Worchester, MA 01655

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14. ABSTRACT
Melanoma is the most aggressive and lethal form of skin cancer. In 2013 over 75,000 Americans were diagnosed with melanoma, and nearly 10,000 died from this disease. It has been known for over a decade that mutations that overactivate the *BRAF* and *NRAS* genes promote melanoma formation. At the same time it has also become clear that these mutations are not sufficient for melanoma formation and other genes are involved. Using genomic studies and cross-species comparisons, we identified the BMP factor *GDF6* as a gene that may cooperate with mutant *BRAF* to promote melanoma. The aims of this grant are to determine if *GDF6* does in fact cooperate with mutant *BRAF* and uncover the mechanisms by which *GDF6* acts in melanomas and normal melanocytes. Toward these aims, we have used our zebrafish model to demonstrate cooperativity between *GDF6* and mutant *BRAF* in accelerating melanoma onset. Furthermore, we have knocked down *GDF6* in human melanoma cells, finding that loss of *GDF6* causes cells to cease proliferating. These and other data suggest that *GDF6* promotes melanoma progression and its withdrawal is detrimental to melanoma cell growth. We are currently investigating whether blocking *GDF6* function is a viable therapeutic strategy.

15. SUBJECT TERMS : nothing listed

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Table of Contents

INTRODUCTION:

Melanoma is the most aggressive skin cancer, and every year it kills nearly 10,000 Americans and roughly 60,000 people worldwide. A greater understanding of the genetic basis for melanoma is essential for designing new ways to diagnose and treat this disease. Nearly a decade ago, it was discovered that mutations that inappropriately activate the *BRAF* gene are present in over half of all human melanomas. Activated *BRAF* mutations are necessary for formation of these melanomas, but numerous studies have shown that they are not sufficient. To find other genes that cooperate with *BRAF* in creating melanomas, we have used genomic studies and cross-species comparisons to identify several candidates. One of these candidates, *GDF6*, is a BMP factor that is recurrently amplified and upregulated in human and zebrafish melanomas. The purpose of this study is to functionally analyze the role of *GDF6* in melanoma progression. In addition, this study aims to use gain and loss of function studies to determine how *GDF6* acts in melanomas and normal melanocytes. A major goal of this research is to determine if *GDF6* can be used as a diagnostic or prognostic marker in melanoma and is a potential therapeutic target.

BODY:

As requested in the Technical Reporting Requirements, this section describes research progress in reference to each task outlined in the Statement of Work. Below, I restate each task and briefly describe its components. With each task an update on progress made is included.

Task 1: Perform gain and loss of function studies in zebrafish embryos and mammalian cultured cells to determine if GDF6 antagonizes melanocyte development.

In this task, studies in zebrafish were proposed to determine the effects of *gdf6b* overexpression and *gdf6b* loss on melanocyte development. With respect to overexpression, we have used the 'MiniCoopR' assay[1,2] to generate animals that overexpress *gdf6b* in the melanocyte lineage, including in melanocyte progenitor cells. As compared to control animals that express a truncated version of *gdf6b*, these animals fail to generate melanocytes. We are currently making a stably transgenic line that overexpresses *gdf6b* to further these studies. For *gdf6b* loss of function studies, strains containing *gdf6b* mutations have been generated. These strains are currently being bred into backgrounds suitable for studying melanocyte development and melanoma progression. Experiments to overexpress *GDF6* in cultured human cells have commenced. As part of these experiments, DNA clones of *GDF6* have been generated using TALEN-mediated mutagenesis[3], transfections into human primed melanocytes and melanoma cells performed, and lines stably expressing *GDF6* created.

Task 2: Use established screening procedures in zebrafish to determine if GDF6 overexpression accelerates melanoma onset or exacerbates other properties of melanomas. In addition, use human melanoma cells to determine if GDF6 knockdown in GDF6-positive cells or overexpression in GDF6-minus cells affects tumorigenicity.

A major part of this project is to assess whether *GDF6/gdf6b* has an effect on melanoma progression. The first part of this task is meant to address this issue by asking whether *gdf6b*-expressing melanocytes give rise to melanomas more quickly than do melanocytes expressing a control *EGFP* gene. Animals with such melanocytes were generated, and we found that *gdf6b*-expressing melanocytes gave rise to melanomas more quickly than those expressing *EGFP* (Fig. 1). Next generation sequencing was used to compare RNA from *gdf6b*-expressing versus *EGFP*-expressing tumors. Differential expression analysis was performed, suggesting that *gdf6b* may promote melanoma formation by protecting melanocytes or nascent melanoma cells from programmed cell death. This model is currently being tested in zebrafish and tissue culture studies.

Another part of this task was to determine if *GDF6* contributes to growth and maintenance of cultured melanoma cells. Toward this end, *GDF6* was knocked down in several melanoma cell lines using multiple anti-*GDF6* shRNA constructs. Knockdown of *GDF6* caused melanoma cells to fail to proliferate in both MTT and clonogenic growth assays (Fig. 2). Preliminary results suggest that this failure is due to programmed cell death. This failure is specific since addition of recombinant, purified GDF6 protein rescued the proliferation defect (Fig. 3). Rescue was achieved when media was supplemented with the secreted variant of GDF6, suggesting that GDF6 acts as a ligand to promote the growth of melanoma cells. Although not part of the original grant application, we are currently investigating the possibility of blocking secreted GDF6 protein as a therapeutic strategy.

The final part of this task was to infect primed HMEL468 melanocytes[4] with virus to generate cells that express *GDF6* at high levels. These melanocytes have been generated stable cell lines selected. Currently we are assessing whether *GDF6*-expressing primed melanocytes have a growth advantage over control melanocytes.

Task 3: Use BMP pathway reporters to determine the dynamics of BMP activity in normal melanocytes and melanoma cells. Examine GDF6 expression and mutation status in human melanomas, benign melanocytic lesions and normal melanocytes to determine if modulation of GDF6 activity is consistent with a role in melanoma formation.

The major goal of this task is to assess whether increases in GDF6 correlate with increases in BMP signaling pathway activity. In the first part of this task, the activity of BMP reporters in zebrafish melanomas will be measured. Currently, stable transgenic lines of a BMP reporter in a melanoma-prone background have been generated. In the next generation of animals it will be possible to measure BMP activity in melanomas. In addition a strain has been generated for the purpose of measuring BMP activity in *GDF6*-expressing melanomas.

Another component of this task is to measure BMP pathway activity in human melanomas. In collaboration with Dr. April Deng of the University of Massachusetts Medical School Department of Dermatopathology, we have stained human melanomas and melanocytic lesions for GDF6 protein. GDF6 protein was present in melanocytic nevi and melanomas but not normal melanocytes (Fig. 4). GDF6 is also expressed in cultured melanoma cell lines (Fig. 5). In these cultured cells, knockdown of *GDF6* led to diminished levels of phosphorylated-SMAD1/5/8 and phosphorylated-p38, both of which are downstream indicators of BMP pathway activity. These data further support the notion that BMP signaling is aberrantly activated by GDF6 in melanomas.

KEY RESEARCH ACCOMPLISHMENTS:

- Determined that high expression of *GDF6* promotes melanoma initiation.
- Performed knockdown experiments to show that loss of *GDF6* in human melanoma cells caused failure of these cells to proliferate.
- Found that failure of proliferation caused by *GDF6* knockdown can be rescued by recombinant GDF6 protein, suggesting that GDF6 acts as a secreted protein and may be targeted in vivo by antibody or other biological therapies.
- Stained human benign and malignant melanocytic lesion sections and determined that GDF6 protein is expressed highly in melanomas, at lower levels in melanocytic nevi and absent from normal melanocytes. Phosphorylated-SMAD1/5/8 and phosphorylated p38, markers of BMP pathway activity are present in melanomas, suggesting that GDF6 acts via these pathways to promote melanoma growth.
- Generated several reagents to support future GDF6 studies, including *gdf6b* mutant zebrafish strains, *GDF6*-overexpressing melanoma cell lines, and *GDF6*-knockdown cell lines.
- Performed next-generation sequencing of normal and *gdf6b*-expressing zebrafish melanomas. These data are currently being analyzed to investigate the mechanism of *GDF6* action in melanoma.

REPORTABLE OUTCOMES:

Presentations during this reporting period include:

- University of Michigan, Molecular, Cellular and Developmental Biology Seminar
- University of Massachusetts Dartmouth, Biology and Bioengineering Seminar
- 7th Zebrafish Disease Models Conference, selected talk
- 22nd International Pigment Cell Conference, selected talk

Cell lines created during this reporting period include:

- GDF6-overexpressing melanoma cell lines
- GDF6-knockdown melanoma cell lines

Zebrafish strains created during this reporting period include:

- Strains with loss-of-function mutations in *GDF6*
- Strains with *GDF6* overexpression in melanocytes

CONCLUSION:

During this reporting period we have obtained results to support the hypothesis that the BMP factor *GDF6* promotes melanoma initiation and maintenance. Stainings of human melanoma sections indicate that GDF6 protein is present in these lesions but not in normal melanocytes. In cultured melanoma cells knockdown of *GDF6* retards the growth of these cells and likely causes cell death. Overexpression of *gdf6b* in our zebrafish model promotes the initiation of melanomas. Taken together, these and other data suggest that *GDF6* is a novel melanoma gene.

Why do these findings regarding *GDF6* matter? Our data indicate that *GDF6* promotes melanoma, and its knockdown impairs melanoma growth. *GDF6* encodes a protein that is processed to yield a shorter, secreted version of GDF6, which is the active form of this protein[5]. Our analyses show that secreted GDF6 can promote growth of cultured melanoma cells. Secreted GDF6 is potentially an important therapeutic target, one that can be inhibited not only by small molecules but also by 'biologic' therapies that can act without having to cross cell membranes. Antibodies, such as the VEGF blocker bevacizumab, epitomize this type of therapy. We are currently investigating the possibility that blocking secreted GDF6 with antibodies can be an effective anti-melanoma strategy.

REFERENCES:

1. C.J. Ceol, Y. Houvras, et al., The histone methyltransferase SETDB1 is recurrently amplified in melanoma and accelerates its onset. *Nature*. **471**, 513-517 (2011).
2. S. Iyengar, Y. Houvras, C.J. Ceol, Screening for melanoma modifiers using a zebrafish autochthonous tumor model. *J. Vis. Exp.* **69**, e50086 (2012).
3. T. Cermak, E.J. Doyle, et al., Efficient design and assembly of custom TALEN and other TAL effector-based constructs for DNA targeting. *Nuc. Acids Res.* **39**, e82 (2011).
4. L.A. Garrraway, H.R. Widlund, et al., Integrative genomic analyses identify MITF as a lineage survival oncogene amplified in malignant melanoma. *Nature*. **436**, 117-122 (2005).
5. C.C. Rider, B. Mulloy, Bone morphogenetic protein and growth differentiation factor cytokine families and their protein antagonists. *Biochem J.* **429**, 1-12 (2010).

APPENDICES:

Please see appended *curriculum vitae* for Dr. Ceol.

CRAIG JOSEPH CEOL
Assistant Professor
Program in Molecular Medicine and Program in Cell and Developmental Dynamics
Department of Cancer Biology, University of Massachusetts Medical School
Albert Sherman Center, AS6.1041, 368 Plantation Street, Worcester, MA 01605
Email: Craig.Ceol@umassmed.edu
Date prepared: June 25, 2014

EDUCATION

Yale University, New Haven, CT	1989-1993
B.S./M.S. combined degree in Molecular Biophysics and Biochemistry	
Research Advisor: Dr. Lynne Regan	
Massachusetts Institute of Technology, Cambridge, MA	1995-2003
Ph.D. degree in Biology	
Research Advisor: Dr. H. Robert Horvitz	
Massachusetts Institute of Technology, Cambridge, MA	2003-2004
Postdoctoral Fellow, Department of Biology, HHMI	
Research Advisor: Dr. H. Robert Horvitz	
Harvard Medical School, Children's Hospital Boston, Boston, MA	2004-2008
Postdoctoral Fellow, Division of Hematology/Oncology, HHMI	
Research Advisor: Dr. Leonard I. Zon	

PROFESSIONAL EXPERIENCE

Research Associate, Eli Lilly and Company	1993-1995
Division of Bioproduct Development	
Instructor, Harvard Medical School, Children's Hospital Boston,	2008-2009
Division of Hematology/Oncology	
Assistant Professor, University of Massachusetts Medical School	2010-
Program in Molecular Medicine and Program in Cell Dynamics	
Department of Cancer Biology	

HONORS AND AWARDS

Yale University:	
B.S./M.S. four-year degree, Molecular Biophysics and Biochemistry	1991-1993
Yale University Summer Study Grant	1991
Distinction in Molecular Biophysics and Biochemistry	1993
Massachusetts Institute of Technology:	
Koch Predoctoral Research Fellow	1999-2000
Children's Hospital Boston and Harvard Medical School:	
Damon Runyon Cancer Research Foundation Postdoctoral Fellowship	2005-2007
American Cancer Society Postdoctoral Fellowship (declined)	2005
Winner, Poster prize, Keystone Symposium, Santa Fe, NM	2006
Advances in the Understanding and Treatment of Melanoma	
Winner, Presentation prize, Harvard Stem Cell Institute Symposium	2008
Charles A. King Trust of The Medical Foundation Postdoctoral Fellowship	2008-2009
NIH Pathway to Independence Award (K99/R00), NIAMS	2009-2013
University of Massachusetts Medical School:	
Worcester Foundation for Biomedical Research Award	2011-2012
American Cancer Society Research Scholar Award	2012-2016
Kimmel Scholar Award	2013-2015
Department of Defense Cancer Career Development Award	2013-2015

FUNDING

Active:

RSG-12-150-01-DDC Research Scholar Award, American Cancer Society, Ceol (PI)
Epigenetic determinants of melanoma initiation and maintenance.

R01AR063850-01 NIH/NIAMS, Ceol (PI)
Use of comparative oncogenomics to identify novel regulators of melanoma progression.

CA120099 Dept of Defense Peer Reviewed Cancer Career Development Award, Ceol (PI)
Uncovering the role of BMP signaling in melanocyte development and melanoma tumorigenesis.

SKF-13-123 Kimmel Scholar Award, Ceol (PI)
Mechanisms underlying melanoma initiation and maintenance.

Concluded:

R00AR056899-04 Pathway to Independence Award, NIH/NIAMS, Ceol (PI)
Identifying events and genetic regulators of melanoma progression

P60016170000122 Worcester Foundation for Biomedical Research, Ceol (PI)
Use of comparative genomics to identify oncogenes.

Scientific Meeting Grant, The Company of Biologists, Ceol (PI)
Zebrafish Disease Models 7 conference, Madison, WI, June 28-July 1, 2014

TEACHING AND MENTORING

Teaching:

M.D./Ph.D. Research Tutorial, one discussion group (3hr).	2010
Ph.D. Summer RAPS (Reading, Analysis, Problem Solving paper review), one discussion group (2hr).	2010
Cancer Biology, one lecture (2hr), one discussion group (2hr).	2010-
Molecular Biology of the Cell Cycle, one lecture (0.5hr), one discussion group (2hr)	2011
Stem Cell and Regenerative Biology. Co-coordinator, two lectures and discussion groups (4hr) plus organizational responsibilities.	2011-2012
RAPS, Block II (2hr).	2011-
Topics in Molecular Medicine, one lecture and discussion group (2hr).	2012

Advisory and supervisory responsibilities:

Name	Position	Year(s)	
Rajesh Vyas	Postdoctoral Fellow	2014-	
Fang Liu	Postdoctoral Fellow	2013	
Corrie Painter	Postdoctoral Fellow	2012-	CRI Irvington Inst. Fellow
Ana Neto	Postdoctoral Fellow	2011-	NRSA Fellow, NCI
Sharanya Iyengar	Graduate Student	2010-	
James Neiswender	Graduate Student	2010-	
Arvind Venkatesan	Graduate Student	2011-	
Eli Freiman	Medical Student	2012	
Alec Gramann	Rotating MD/PhD Student	2014	
Tyler Frantz	Rotating MD/PhD Student	2014	
Ciearra Smith	Rotating Graduate Student	2014	
Revati Darp	Rotating Graduate Student	2013	
Heather Kolpa	Rotating Graduate Student	2010	
Jennifer Maurer	Rotating Graduate Student	2010	
James Ritch	Rotating Graduate Student	2010	
Lin Lin	Rotating Graduate Student	2010	
Justin Peter Hess	Undergrad. Student (WPI)	2012	
Sukanya Murali	Undergrad. Student	2013	

(Anna University – Chennai)
Brittney Logan Undergrad. student 2013
(W. New England University)

Dissertation committees:
Shawna Guillemette, UMass Medical School, Cancer Biology Program
Tomoko Tabuchi, UMass Medical School, Interdisciplinary Graduate Program
David Driscoll, UMass Medical School, Cancer Biology Program
Anna Malinkevich, UMass Medical School, Interdisciplinary Graduate Program
Cheng Chang, UMass Medical School, Cancer Biology Program (Chair)
Nomeda Girnius, UMass Medical School, Cancer Biology Program
Lin Lin, UMass Medical School, Cancer Biology Program (Chair)
James Ritch, UMass Medical School, Interdisciplinary Graduate Program
Nicola Kearns, UMass Medical School, Interdisciplinary Graduate Program

Qualifying examination committees:
Christopher Clark, UMass Medical School, Neuroscience Program
Caitlin Fogarty, UMass Medical School, MD/PhD Program
Nomeda Girnius, UMass Medical School, Interdisciplinary Graduate Program
Chien-Min Hung, UMass Medical School, Interdisciplinary Graduate Program
James Ritch, UMass Medical School, Interdisciplinary Graduate Program
Lin Lin, UMass Medical School, Cancer Biology Program (Chair)
Ly-She Ee, UMass Medical School, Interdisciplinary Graduate Program
Shubham Dutta, UMass Medical School, Interdisciplinary Graduate Program
Nicola Kearns, UMass Medical School, Interdisciplinary Graduate Program
Hsi-Ju Chen, UMass Medical School, Interdisciplinary Graduate Program

SERVICE

University of Massachusetts Medical School and local:

Sherman Center Labs NTI/GTC/CVC/Diabetes Focus Group	2010
Diabetes and Endocrinology Research Center (grant reviewer, ad hoc)	2011
AP Biology High School Outreach Program (host)	2011-
University of Massachusetts Medical School Convocation (Dinner and Dialogue event speaker and panelist)	2011
University of Massachusetts Medical School visit of Young President's Organization & World President's Organization (speaker)	2011
University of Massachusetts Medical School Development Council meeting (speaker)	2012
University of Massachusetts Medical School BARG Organization (speaker)	2012
LCME accreditation of University of Massachusetts Medical School (Junior Faculty cohort)	2012
University of Massachusetts Chancellor's Review (Faculty Review Committee)	2012
Wachusett High School Science Seminar	2012
University of Massachusetts Medical School Science to Trades Seminar	2013
MassAHEC Network Frontiers in Science Seminar	2014
NIH BEST Award Focus Group	2014
Hudson Hoagland Society annual meeting (speaker)	2014

Referee for journals:
Molecular and Cellular Oncology – Peer Review Board 2014-
PLoS Genetics – ad hoc 2009-
Proceedings of the National Academy of Sciences USA – ad hoc 2009-
Molecular and Cellular Biology – ad hoc 2010-
PLoS Biology – ad hoc 2011-
FASEB Journal – ad hoc 2012-
Experimental Cell Research – ad hoc 2012-

Genome Research – ad hoc 2012-
Journal of Investigative Dermatology – ad hoc 2012-
Journal of Visualized Experiments – ad hoc 2013-
Cell Death and Differentiation – ad hoc 2013-
Disease Models and Mechanisms – ad hoc 2013-
Cell Death and Disease – ad hoc 2014-

Grant review and study section service:
National Centre for the Replacement, Refinement and Reduction of Animals in Research (Ad Hoc Reviewer) - 2010
University of Massachusetts Medical School Diabetes and Endocrinology Research Center (Ad Hoc Reviewer) - 2010
Association for International Cancer Research (Ad Hoc Reviewer) - 2011
NIH, Cancer Genetics Study Section (CG) (Ad Hoc Reviewer) - 2012
Children's Tumor Foundation - 2013
Medical Research Council (United Kingdom) (Ad Hoc Reviewer) - 2013

Society memberships:
Society for Melanoma Research, 2009-
American Society for Cell Biology, 2012-
American Association for Cancer Research, 2012-
Zebrafish Disease Models Society, 2014-

Meetings and community service:
Co-organizer, Zebrafish Disease Models 7, Madison, Wisconsin, 2014
Co-chair, Cancer Working Group, Zebrafish Disease Models Society, 2014-

PUBLICATIONS

Original reports:

1. **Ceol, C.J.** and Horvitz, H.R. (2001). *dpl-1* DP and *efl-1* E2F act with *lin-35* Rb to antagonize Ras signaling in *C. elegans* vulval development. *Mol. Cell*. *7*, 461-73.
   ‡ This paper is highlighted by the Faculty of 1000.
2. Thomas, J.H.*, **Ceol, C.J.***, Schwartz, H.T. and Horvitz, H.R. (2003). New genes that interact with *lin-35* Rb to negatively regulate the *let-60 ras* pathway in *Caenorhabditis elegans*. *Genetics*. *164*, 135-51.
3. **Ceol, C.J.** and Horvitz, H.R. (2004). A new class of *C. elegans* synMuv genes implicates a Tip60/NuA4-like HAT complex as a negative regulator of Ras signaling. *Dev. Cell*. *6*, 563-76.
   ‡ This paper is highlighted by the Faculty of 1000.
4. **Ceol, C.J.**, Stegmeier, F., Harrison, M.M. and Horvitz, H.R. (2006). Identification and classification of genes that act antagonistically to *let-60* Ras signaling in *Caenorhabditis elegans* vulval development. *Genetics*. *173*, 709-26.
5. Harrison, M.M., **Ceol, C.J.**, Lu X. and Horvitz, H.R. (2006). Some *C. elegans* class B synthetic multivulva proteins encode a conserved LIN-35 Rb-containing complex distinct from a NuRD-like complex. *Proc. Natl. Acad. Sci. USA*. *103*, 16782-7.
6. White, R.M., Sessa, A., Burke, C., Bowman, T., LeBlanc, J., **Ceol, C.J.**, Bourque, C., Dovey, M., Goessling, W., Burns, C.E. and Zon. L.I. (2008). Transparent adult zebrafish as a tool for in vivo transplantation analysis. *Cell Stem Cell*, *2*, 183-9.
7. Langenau, D.M., Keefe, M.D., Storer, N.Y., Jette, C.A., Smith, A.C., **Ceol, C.J.**, Bourque, C., Look, A.T. and Zon, L.I. (2008). Coinjection strategies to modify radiation sensitivity and tumor initiation in transgenic zebrafish, *Oncogene*, *27*, 4242-8.
8. Goessling, W., North, T.E., Lord, A.M., **Ceol, C.J.**, Weidinger, G., Lee, S., Strijbosch, R., Haramis, A., Puder, M., Clevers, H., Moon, R.T. and Zon, L.I. (2008). APC mutant zebrafish uncover a changing temporal requirement for wnt signaling in liver development. *Dev. Biol.*, *320*, 161-74.
9. Freeman, J.L., **Ceol, C.J.**, Feng, H., Langenau, D.M., Belair, C., Stern, H.M., Song, A, Paw, B.H., Look, A.T., Zhou, Y., Zon, L.I. and Lee, C. (2009). Construction and application of a cytogenetically-validated zebrafish-specific array CGH platform. *Genes Chromosomes Cancer*, *48*, 155-70.

10. North, T.E., Goessling, W., Peeters, M., Li, P., **Ceol, C.J.**, Lord, A.M., Weber, G.J., Harris, J., Cutting, C.C., Huang, P., Dzierzak, E., Zon, L.I. (2009). Hematopoetic stem cell development is dependent on blood flow. *Cell*, *137*, 436-48.
11. **Ceol, C.J.***, Houvras, Y.*, Jane-Valbuena, J., Bilodeau, S., Orlando, D., Battisti, V., Fritsch, L., Lin, W., Hollmann, T.J., Ferré, F., Bourque, C., Burke, C., Turner, L., Uong, A., Johnson, L.A., Beroukhim, R., Mermel, C., Loda, M., Ait-Si-Ali, S., Garraway, L., Young R.A. and Zon, L.I. (2011). The *SETDB1* histone methyltransferase is recurrently amplified in and accelerates melanoma. *Nature*, *471*, 513-7.
12. Richardson, J., Zeng, Z., **Ceol, C.J.**, Jackson, I.J., Patton, E.E. (2011). *BRAF*^{V600E} nevi regenerate from an undifferentiated precursor population in zebrafish. *Pigment Cell Melanoma Research*, *24*, 378-81.
13. Lian, C.G., Xu. Y., **Ceol, C.J.**, Wu, F., Larson, A., Dresser, K., Xu, W., Tan, L., Zhan, Q., Lee, C., Hu, D., Lian, B.Q., Kleffel, S., Yang, Y., Khorasani, A.J., Lezcano, C., Duncan, L.M., Scolyer, R.A., Thompson, J.F., Kakavand, H., Houvras, Y., Zon, L., Mihm Jr., M.C., Kaiser, U.B., Schatton, T., Woda, B.A., Murphy, G.F. and Shi, Y.G. (2012). Loss of 5-hydroxymethylcytosine is an epigenetic hallmark of melanoma. *Cell*, *150*, *1135-46*.
    ‡ This paper is highlighted by the Faculty of 1000.
14. Iyengar, S., Houvras, Y. and **Ceol, C.J.** (2012). Screening for melanoma modifiers using a zebrafish autochthonous tumor model. *Journal of Visualized Experiments*, *69, e50086*.
15. Painter, C.A. and **Ceol, C.J.** (2014). Zebrafish as a platform to study tumor progression. *Methods in Molecular Biology*, in the press.

Reviews and commentary:

1. **Ceol, C.J.**, Pellman D. and Zon, L.I. (2007). APC and colon cancer: two hits for one. *Nat. Med*. *13*, 1286-7.
2. **Ceol, C.J.***, Houvras, Y.*, White R.M.* and Zon, L.I. (2008). Melanoma biology and the promise of zebrafish. *Zebrafish* *5*, 247-55.
3. **Ceol, C.J.** (2011). Acta Eruditorum: Certain genes accelerate melanoma development. *Dermatology World* *21*, 11-12.

Cover art:

Ceol, C.J.*, Houvras, Y.*, Jane-Valbuena, J., Bilodeau, S., Orlando, D., Battisti, V., Fritsch, L., Lin, W., Hollmann, T.J., Ferré, F., Bourque, C., Burke, C., Turner, L., Uong, A., Johnson, L.A., Beroukhim, R., Mermel, C., Loda, M., Ait-Si-Ali, S., Garraway, L., Young R.A. and Zon, L.I. (2011). The *SETDB1* histone methyltransferase is recurrently amplified in and accelerates melanoma. *Nature*, *471*, 513-7.

ORAL PRESENTATIONS

Meeting presentations:

East Coast *C. elegans* Meeting, Boston, MA	1998
International *C. elegans* Meeting, Madison, WI	1999
East Coast *C. elegans* Meeting, Durham, NH	2002
Keystone Symposium, *Advances in the Understanding and Treatment of Melanoma*, Santa Fe, NM	2006
Gordon Conference, *Cancer Models and Mechanisms*, Les Diablerets, Switzerland	2008
8th International Conference on Zebrafish Development and Genetics, Madison, WI	2008
Harvard Stem Cell Institute Research Symposium, Boston, MA	2008
9th International Conference on Zebrafish Development and Genetics, Madison, WI	2009
3rd Zebrafish Disease Models Conference, Boston, MA	2010
Connecticut Valley Zebrafish Meeting, Middletown, CT	2010
Gordon Conference, *Cancer Genetics and Epigenetics*, Ventura, CA	2011
Biotechcellence 2012 National Technical Symposium Anna University, Chennai, India (via videoconference)	2012
10th International Conference on Zebrafish Development and Genetics, Madison, WI (workshop co-coordinator)	2012
International Federation of Pigment Cell Societies, Pigment Cell Development Workshop, Edinburgh, UK	2013
5th European Melanoma Conference, *Basic and clinical research join forces to defeat*	2013

melanoma, Marseille, France
6th Zebrafish Disease Models Conference, Murcia, Spain 2013
7th Zebrafish Disease Models Conference, Madison, Wisconsin 2014
22nd International Pigment Cell Conference, *Bringing colours to life*, Singapore 2014

Invited seminar presentations:
Hubrecht Institute, Utrecht, Netherlands 2008
Cancer Genomics and Developmental Biology Programme Seminar
Whitehead Institute, Massachusetts Institute of Technology, Cambridge, MA 2008
Whitehead Seminar Series for High School Teachers: Contolling Genes
Providence College, Providence, RI 2011
Biology Department Seminar
UMass Medical School, Worcester, MA 2011
Cutaneous Tumor Board, Pathology Department
University of Rochester Medical Center, Rochester, NY 2011
Biomedical Genetics Department Seminar
Quinsigamond Dermatological Society, Worcester, MA 2011
Grand Rounds
Carnegie Institution, Baltimore, MD 2012
Department of Embryology Seminar
National Institutes of Health, Bethesda, MD 2012
NIH Comparative Biomedical Scientist Program Symposium
University of Massachusetts Medical School, Worcester, MA 2012
Cancer Biology Retreat
Assumption College, Worcester, MA 2012
Seminar in Life Sciences
University of Massachusetts Medical School, Worcester, MA 2013
Microbiology and Physiological Systems Department Seminar
Tufts University School of Medicine, Boston, MA 2013
Molecular Physiology and Pharmacology Retreat (Keynote)
Centro Andaluz de Biología del Desarrollo, Seville, Spain 2013
CABD Institute Seminar
University of Michigan, Ann Arbor, MI 2014
Molecular, Cellular and Developmental Biology Seminar
University of Massachusetts, Dartmouth, MA 2014
Biology and Bioengineering Seminar

SUPPORTING DATA:

The following figures are appended:
Figure 1: Expression of *gdf6b* accelerates melanoma onset.
Figure 2: *GDF6* knockdown blocks melanoma proliferation.
Figure 3: *GDF6* knockdown is rescued by recombinant GDF6 ligand.
Figure 4: GDF6 immunohistochemistry.
Figure 5: Knockdown of GDF6 diminishes phosphorylated SMAD1/5/8 and phosphorylated p38.

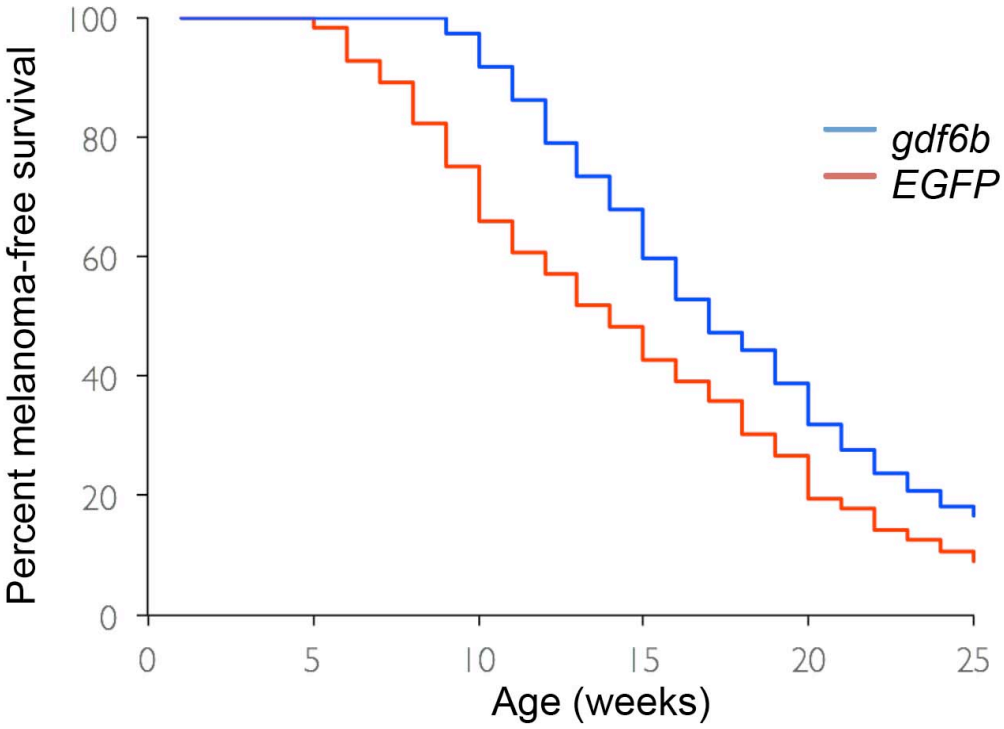

Figure 1. Expression of *gdf6b* accelerates melanoma onset. In this assay melanocytes expressing the zebrafish *GDF6* ortholog *gdf6b* or *EGFP* were reconstituted in a *BRAF*^{V600E}-positive and *p53*-negative background. Transgenic fish were monitored weekly to determine time of melanoma onset. This is an autochthonous tumor model, so even the earliest stages of tumor progression are captured.

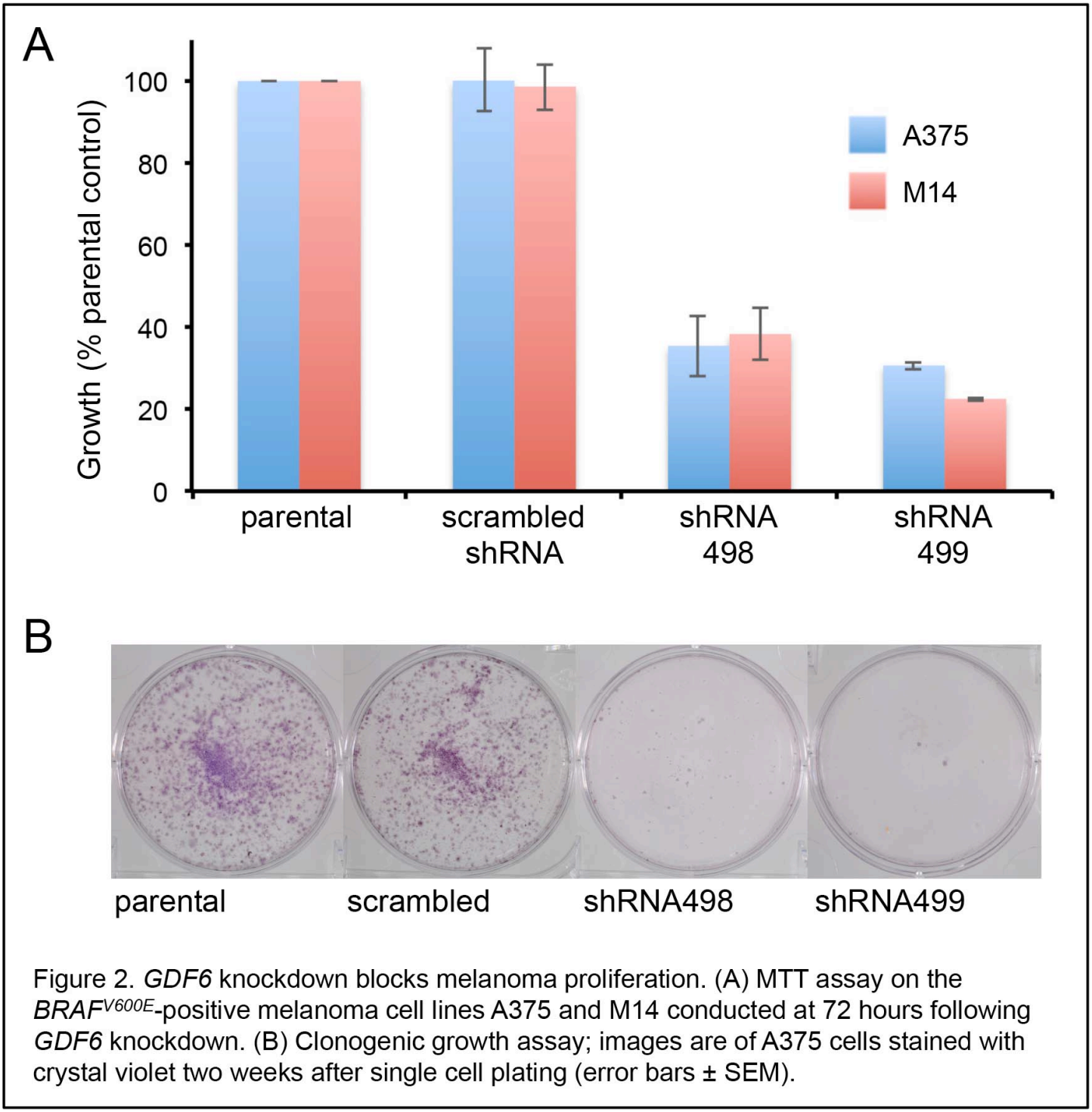

Figure 2. *GDF6* knockdown blocks melanoma proliferation. (A) MTT assay on the *BRAF*^{V600E}-positive melanoma cell lines A375 and M14 conducted at 72 hours following *GDF6* knockdown. (B) Clonogenic growth assay; images are of A375 cells stained with crystal violet two weeks after single cell plating (error bars ± SEM).

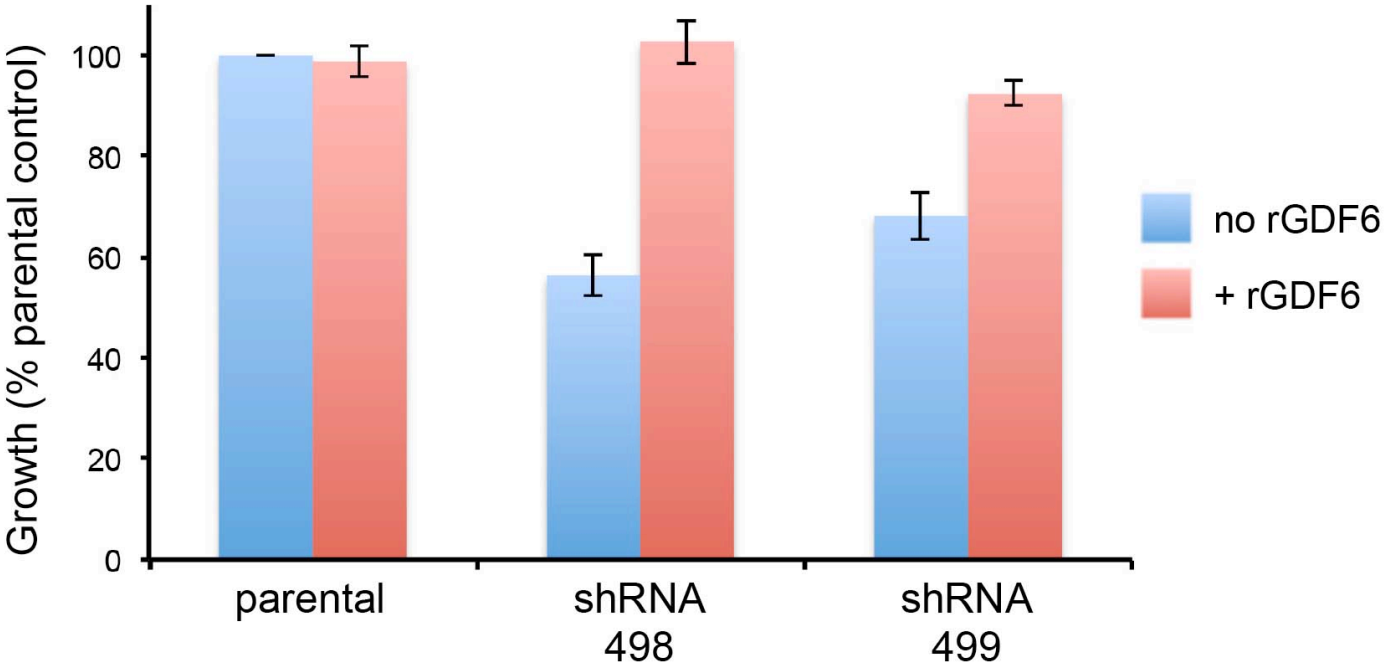

Figure 3. *GDF6* knockdown is rescued by recombinant GDF6 ligand. MTT assays were performed 72 hours following *GDF6* knockdown and supplementation of media with GDF6 ligand or mock supplement (error bars ± SEM).

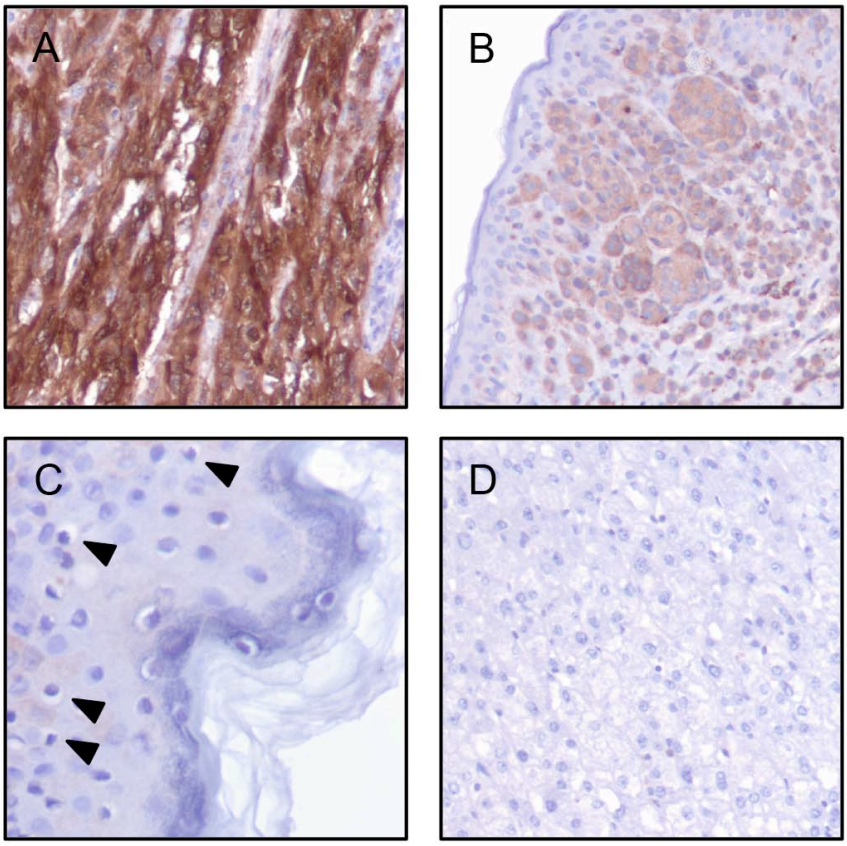

Figure 4. GDF6 immunohistochemistry. (A) Melanoma, (B) Common nevus, (C) Normal skin, and (D) Liver tissue stained with an anti-GDF6 antibody. All images are 200x magnification except (C), which is at 400x. Normal melanocytes are indicated (arrowheads).

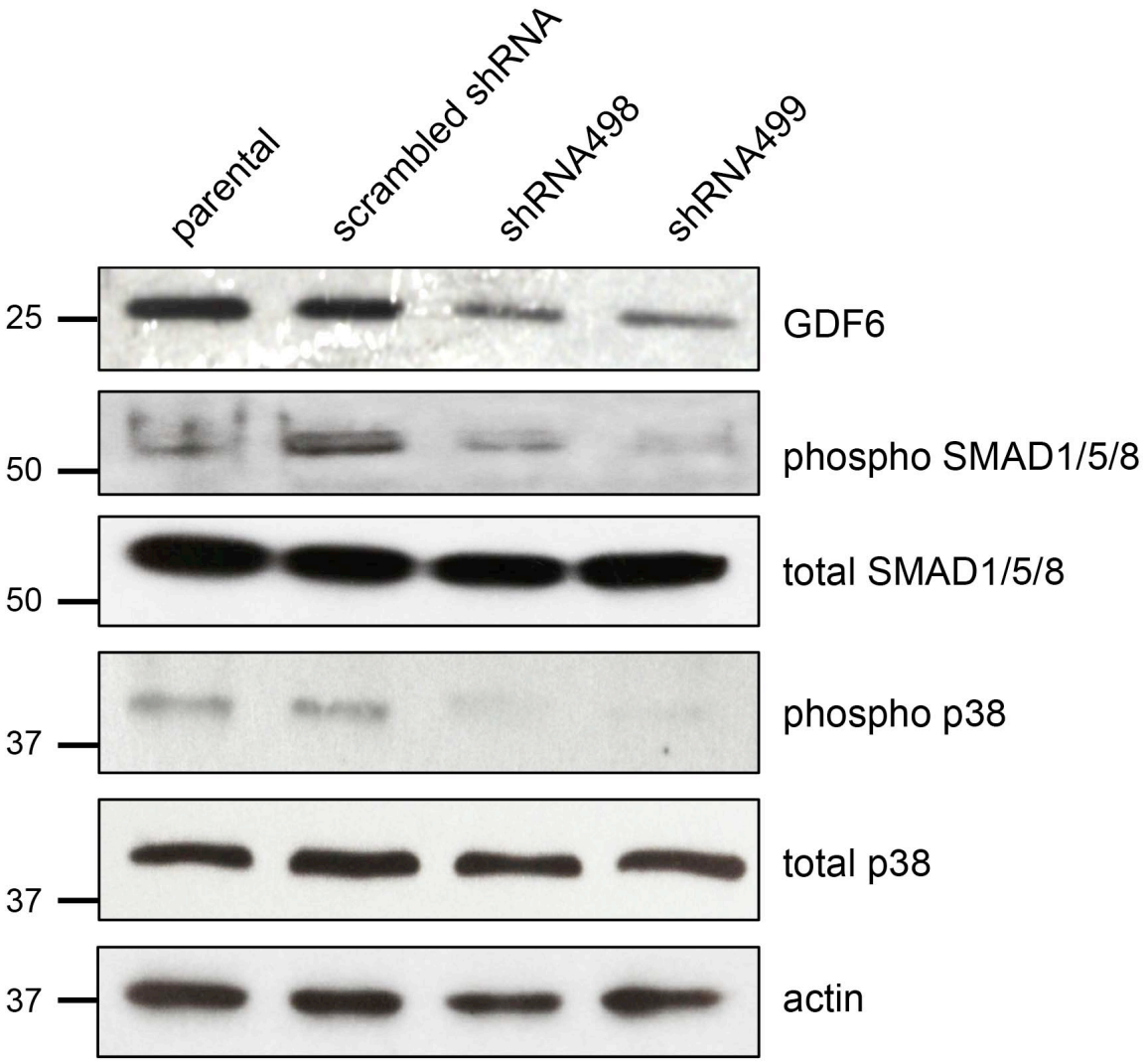

Figure 5. Knockdown of GDF6 diminishes phosphorylated SMAD1/5/8 and phosphorylated p38. Western blots of control and shRNA knockdown M14 cellular lysates. 30µg total protein was loaded per lane, and actin was used as a loading control.